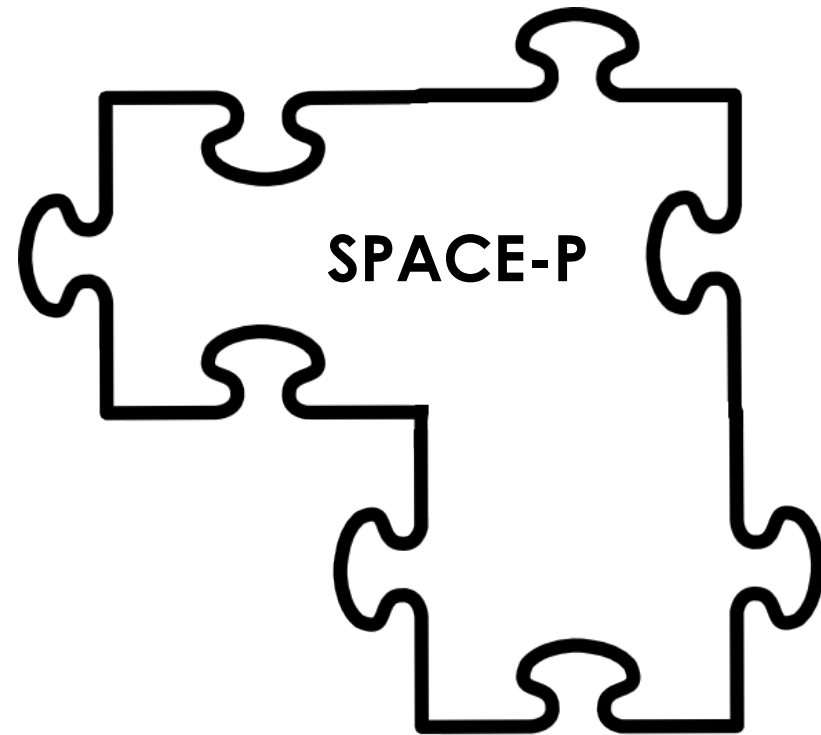
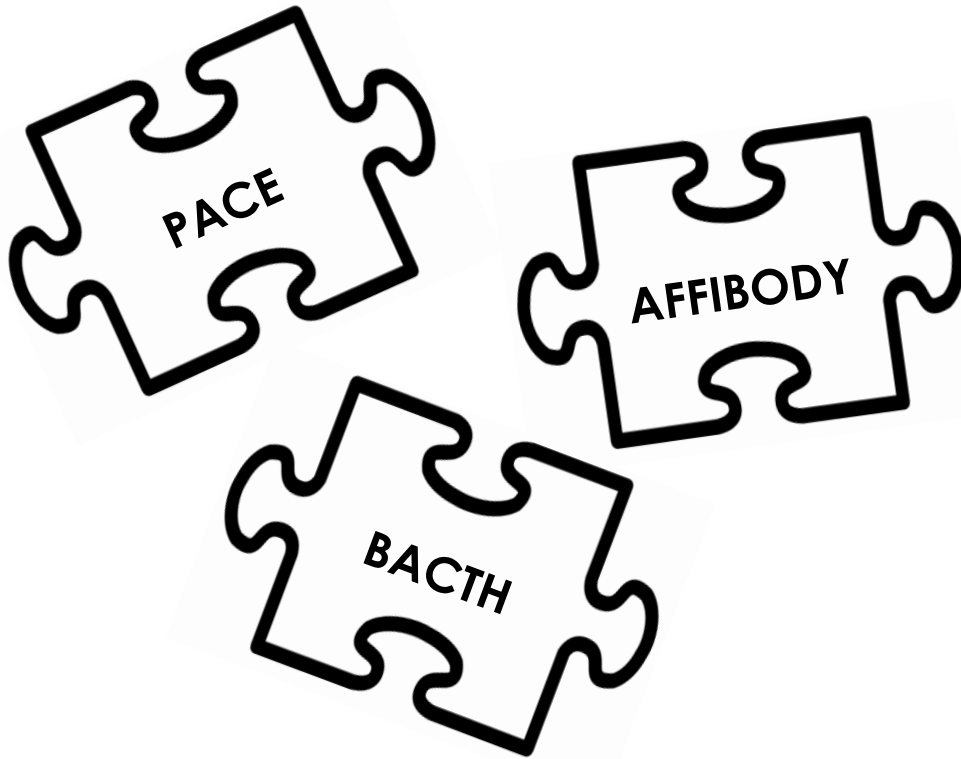


# iGEM 2015: Evolution through time and SPACE-P

Negative selection and stringency modulation in  
phage-assisted continuous evolution

*Carlson (2014)*

# Overview



PACE	Phage Assisted Continuous Evolution
BACTH	Bacterial Adenylate Cyclase Two Hybrid
SPACE-P	Structural Phage Assisted Continuous Evolution of Proteins

# Phage Assisted Continuous Evolution



MP: mutagenesis plasmid  
AP: accessory plasmid

lock

gene III

Selection Phage

"Lagoon"

Non-viable

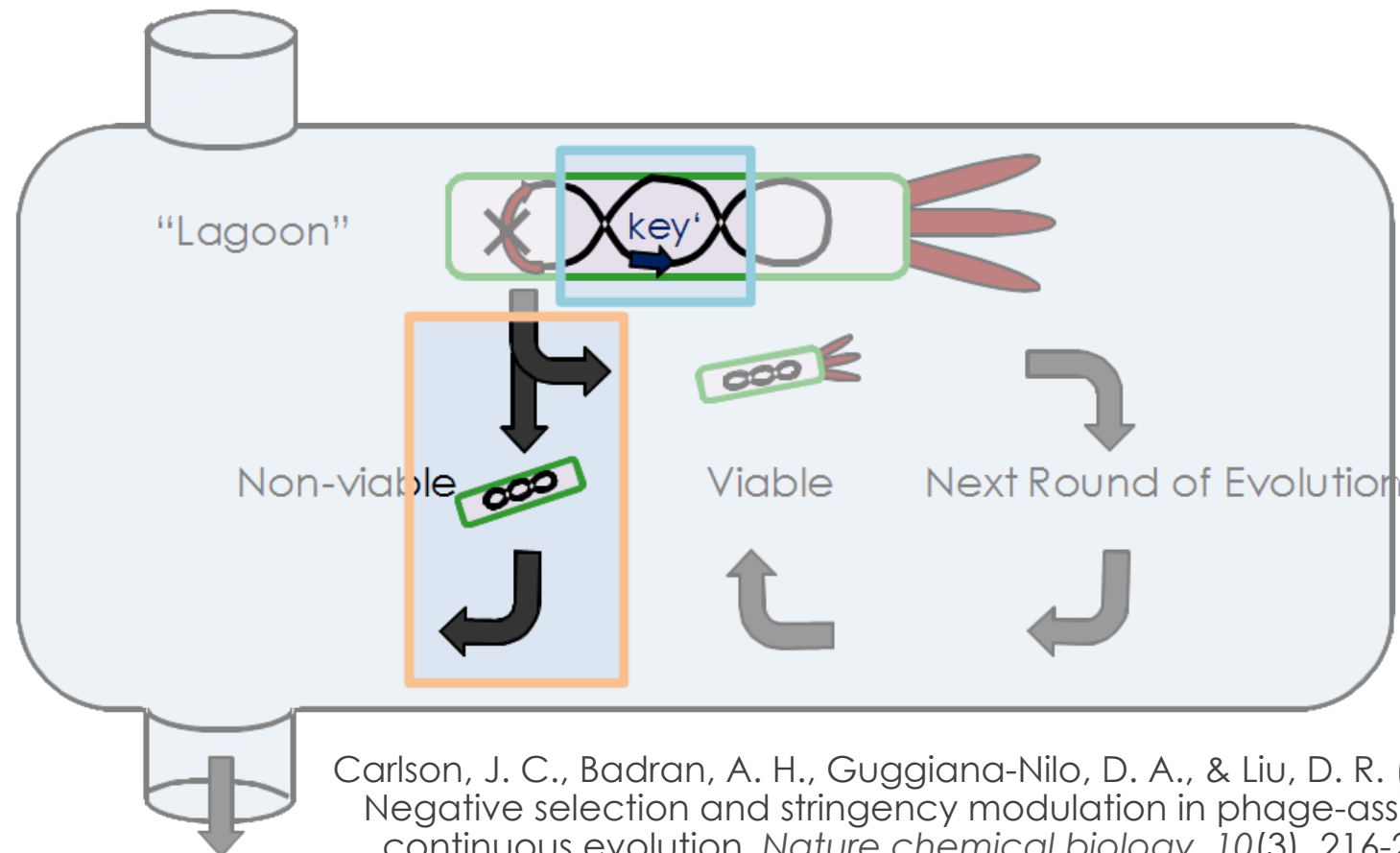
Viable

Next Round of Evolution

Esvelt, K. M., Carlson, J. C., & Liu, D. R. (2011). A system for the continuous directed evolution of biomolecules. *Nature*, 472(7344), 499-503.

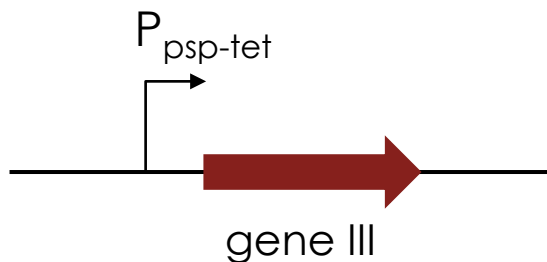
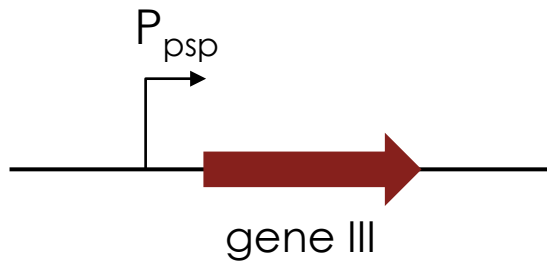
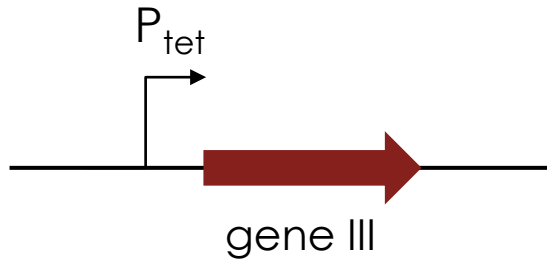
# Challenges

Challenge	Consequence
Inactive key-lock starting pair	Initial washing out of phages
Only positive selection of the key	Broadening of key specificity



Carlson, J. C., Badran, A. H., Guggiana-Nilo, D. A., & Liu, D. R. (2014). Negative selection and stringency modulation in phage-assisted continuous evolution. *Nature chemical biology*, 10(3), 216-222.

# Stringency Modulation



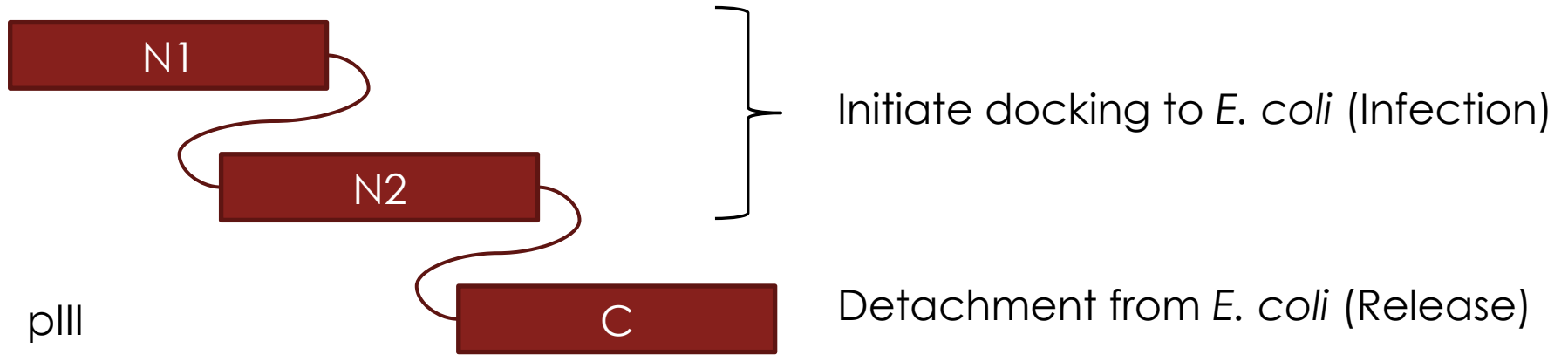
- ATc- dependent
- uninfected cells become resistant to phage infection

- pIV - dependent

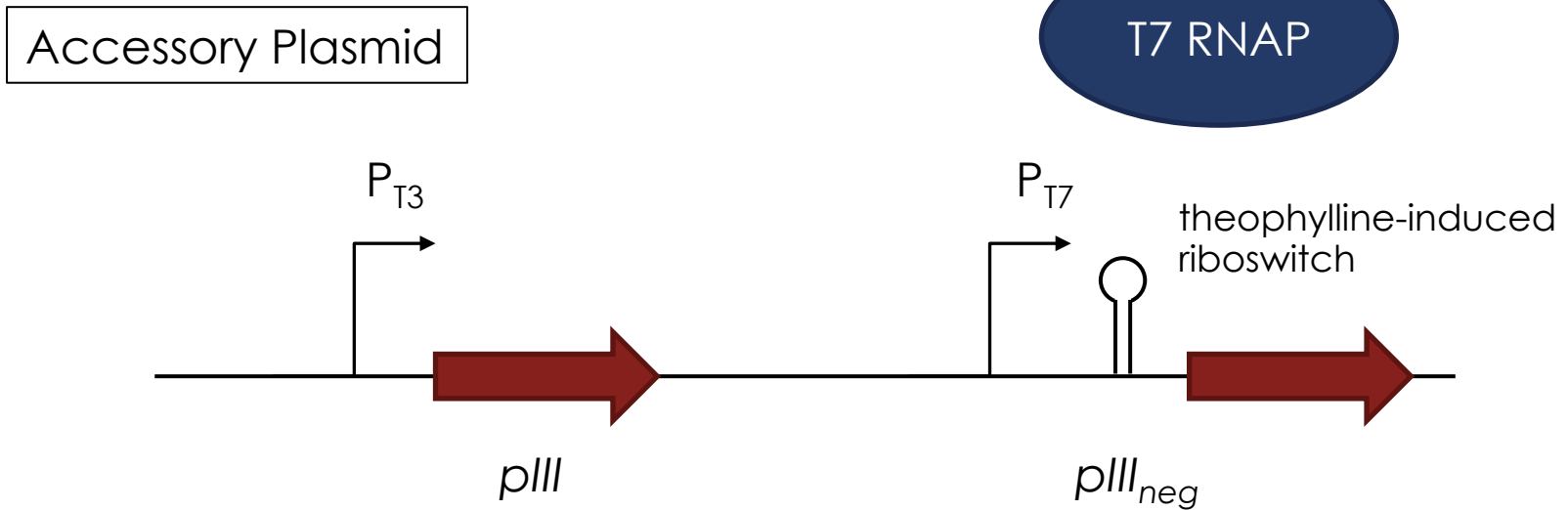
- (pIV + ATc) - dependent

$P_{tet}$	Tetracycline Promoter
$P_{psp}$	Phage Shock Promoter
ATc	Anhydrotetracycline

# Negative Selection



# Setup for Specificity Shift



Step I: broaden substrate specificity ( $pIII$ )

Step II: narrow changed substrate specificity ( $pIII_{neg}$ )

# Power of PACE



- ✓ Evolve proteins directed and continuous
- ✓ Easy method (simple organisms & setup)
- ✓ Fast technique (2-3 days)
- ✓ Stringency modulation possible
- ✓ Negative selection possible